

# Molecular Serotyping of Dengue Virus and Population Impact from an Underreported Region in Southern Bengal, Eastern India: The 2018 Story.

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## Abstract:

**Background:** Dengue virus (DENV) infection is a significant public health concern in India, with regional variations in circulating serotypes. However, molecular surveillance data from underreported zones of Southern Bengal are lacking. This study aimed to identify the distribution of dengue cases and determine the predominant serotypes circulating in this region.

**Materials and Methods:** A total of 110 rapid test-positive patient samples were collected from various Underreported zones of Southern Bengal in 2018. DENV positivity was initially confirmed by NS1 antigen ELISA. Subsequent viral RNA detection and serotyping were performed using real-time PCR assays.

**Results:** Among the total samples, 86.36% (95/110) tested positive for DENV-2 RNA, and 2.72% (3/110) were positive for DENV-3. The highest number of cases was reported from Pingla (46.36%), followed by Debra (19.09%) and Ghatal (17.27%), indicating endemic clusters. Most patients were male (58.18%), and the majority of infections (81.81%) occurred in the 19–59-year age group.

**Conclusion:** This is the first report of molecular serotyping of dengue virus from this underrepresented region of Eastern India. DENV-2 was identified as the predominant serotype, with limited presence of DENV-3. These findings underscore the need for continuous serotype-specific surveillance to inform targeted public health interventions and improve regional outbreak preparedness.

**Key Word:** Dengue virus, Serotyping, DENV-2, Real-time PCR, Southern Bengal, Eastern India, Molecular epidemiology

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## I. INTRODUCTION

Dengue fever remains a significant global public health challenge, particularly in tropical and subtropical regions, where nearly 2.5 billion individuals are at risk. The dengue virus (DENV), a member of the *Flavivirus* genus, is transmitted primarily by *Aedes aegypti*, a day-biting mosquito that thrives in domestic settings and breeds in stagnant water(1).

DENV exhibits considerable genetic diversity, comprising four distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) and multiple genotypes within each serotype (2,3). Reports of dengue outbreaks across various regions of India have increased significantly over the past decade (4). Recent studies suggest that India accounts for approximately 33% of global DENV infections (5,6).

The rapid growth of industries, increased construction activity, expansion of transport networks, and intensified human movement between urban and rural areas, combined with environmental changes, have created favorable ecological conditions for the spread of dengue in rural and urban settings. Notably, all four serotypes have been found to co-circulate, with concurrent infections documented, positioning India as one of the world's hyperendemic regions for dengue (7, 8, 9).

Efficient and early diagnosis of dengue viral infection is therefore essential. Laboratory diagnosis is critical, as clinical identification is often complicated by the wide spectrum of clinical manifestations. Rapid diagnostic test kits have proven to be unreliable in many cases. Although IgM-capture ELISA is widely used to detect anti-dengue antibodies, these assays may fail to detect infection in the early stages of illness or cases of secondary infection. Consequently, detection of the virus-encoded non-structural protein 1 (NS1) antigen has become the preferred diagnostic tool (10). NS1, a glycoprotein essential for viral replication and viability, is typically present at high levels during early viremia. Several assays have been developed to detect NS1 antigenemia, which is often associated with diverse clinical presentations (11). However, variability in the

detection sensitivity during the acute phase of infection has raised concerns regarding the reliability of NS1-based diagnostics.

To address this, we employed real-time PCR-based detection of viral RNA in parallel with the NS1 ELISA assay, allowing for a comparative analysis of these techniques in identifying circulating serotypes(12). Accurate identification and mapping of DENV serotypes in a given region is a key strategy for tracking virus transmission, managing outbreaks, and preventing the escalation of localized epidemics. The aim of our study is to identify the prevalent dengue virus serotypes circulating in and around the southern region of West Bengal and to evaluate associated clinical, demographic, and environmental factors that support the classification of this area as a dengue-prone hot zone.

## **II. MATERIALS AND METHODS**

This prospective study was conducted at Midnapore Medical College and Hospital (MMCH), a tertiary care teaching hospital, in West Midnapore district, West Bengal, India. Before performing this study, Institutional Ethics Committee (IEC) approval was obtained.

**Study Design:** A tertiary care hospital-based observational study.

**Study Location:** This was a hospital-based observational study conducted at the Virus Research and Diagnostic Laboratory, Department of Microbiology, Midnapore Medical College and Hospital — a tertiary care teaching institution in Midnapore, West Bengal, India.

**Study Duration:** January 2018 to December 2018.

**Sample size:** 110 patients.

**Subjects & selection method:** Samples were collected from various zones within West Midnapore, located in Eastern India (latitude 22.3°N, longitude 87.3°E). An exploratory research study was conducted at Midnapore Medical College and Hospital to determine the serotypes of the dengue virus in patients clinically suspected of having dengue fever. Given that this region is dengue-endemic, clinical samples were obtained from both the medicine and paediatric outpatient departments (OPDs), hospitalized patients at Midnapore Medical College and Hospital, as well as individuals attending rural, sub-divisional, and primary health centres across the district. Prominent underreported sample collection sites included Debra, Pingla, and Ghatal.

### **Inclusion criteria:**

1. Patients presenting with an acute fever of 3–5 days duration
2. Either sex
3. Aged 0-80 years,
4. Body temperature of  $\geq 38^{\circ}\text{C}$ , with or without accompanying symptoms such as headache, fatigue, rash, loss of appetite, nausea, vomiting, myalgia, or retro-orbital pain

### **Exclusion criteria:**

1. Patients who declined to provide informed consent
2. Those with a fever attributed to known systemic conditions or complications

**Weather effect:** Weather data were collected from <https://en.climate-data.org/asia/india/west-bengal/medinipur-24458/>. The recorded parameters included average minimum and maximum temperatures as well as average rainfall. These data were analyzed to assess potential correlations between weather patterns and dengue incidence.

**Ethical considerations:** The study was approved by the Institutional Ethics Committee of Midnapore Medical College and Hospital and the review boards of participating health centres. Written informed consent was obtained from all participants or from parents/guardians in the case of individuals under 18 years of age.

### **Procedure methodology**

**Clinical samples:** Peripheral venous blood samples (2–3 mL) were collected from patients presenting with dengue-like symptoms for  $\geq 3$  to  $\leq 5$  days, between January 2018 and December 2018 (13). Blood samples were allowed to clot at room temperature ( $20^{\circ}\text{C}$ – $25^{\circ}\text{C}$ ) and centrifuged at 3300 rpm for 10 minutes (14). Serum samples not tested within 48 hours were aliquoted into sterile vials and stored at  $-80^{\circ}\text{C}$  for later analysis.

Patients were clinically categorized according to World Health Organization (WHO) guidelines based on disease severity and outcomes (15). A total of 110 samples were included in the study, collected from diverse locations within and surrounding West Midnapore. Informed consent was obtained from all participating individuals.

**Antigenic Surveillance:** Serum samples were screened for dengue virus NS1 antigen using a commercially available ELISA kit (InBios International, Inc., USA), following the manufacturer's protocol.

**RNA extraction:** The MagMAX™ Viral/Pathogen II (MVP II) nucleic acid isolation kit was used to extract RNA using the KingFisher™ automated extraction system (Thermo Fisher Scientific), following the manufacturer's instructions. In brief, KingFisher instruments use magnetic beads to selectively bind nucleic acids, thereby automating the RNA extraction process. These beads bind nucleic acids more efficiently than glass-fiber filters, resulting in higher and more consistent yields. The process involves a simple sequence of binding, washing, and elution steps. The KingFisher system can be programmed to extract the desired analyte automatically, and the captured nucleic acids can be subsequently eluted in an elution buffer for use in downstream applications.

**TaqMan Real-Time PCR Assay:** TaqMan-based real-time RT-PCR amplification was performed using the commercially available GENES2ME DENGUE-Q Real-Time PCR Kit (16,17). This kit is designed to detect dengue virus serotypes DENV-1, DENV-2, DENV-3, and DENV-4. The fluorescent probes are labelled with FAM for DENV-1, Texas Red for DENV-2, and CY5 for the internal control in the Primer & Probe Mix-1 tube; and with FAM for DENV-4, Texas Red for DENV-3, and CY5 for the internal control in the Primer & Probe Mix-2 tube. The use of probes linked to fluorescent reporter and quencher dyes allows for the detection of dengue-specific RNA as well as the internal control (IC) in the corresponding detection channels of the BIORAD CFX96 real-time PCR instrument.

Each 20 µL reaction was prepared as follows: 8 µL of extracted RNA sample, 4 µL of One-Step 5X qRT-PCR Master Mix (including buffer), 1 µL of Primer & Probe Mix-1 or Mix-2, 1 µL of Internal Control template, and 6 µL of nuclease-free water.

The thermal cycling conditions were: cDNA synthesis at 55°C for 10 minutes, an initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, and annealing/extension at 60°C for 60 seconds.

A specimen was interpreted as positive for DENV-1, DENV-2, DENV-3, or DENV-4 if the amplification curve crossed the threshold line within 35 cycles ( $C_t < 35$ ).

**Statistical Analysis:** A statistical analysis was conducted based on patient age, categorized into four groups: pediatric (0–≤15 years), younger adults (≥16–≤45 years), older adults (≥46–≤59 years), and geriatric (≥60–≤80 years). Normally distributed continuous variables were summarized using the mean and standard deviation. Data analysis was performed using GraphPad Prism Version 5.0 for Windows (GraphPad Software Inc., San Diego, CA, USA).

### III. RESULT

This study was conducted over a one-year period (January to December 2018). The study population consisted of  $n = 110$  dengue cases (rapid test positive). The highest number of clinical cases was observed in the month of August. All samples were initially validated using the dengue NS1 ELISA. Notably, all samples tested positive for NS1 and were subsequently included in this study for further serotyping analysis.

#### **Clinical Outline:**

This study also recorded key clinical symptoms prior to laboratory confirmation of dengue fever. Among the 110 patients, 100% ( $n = 110$ ) reported a history of fever, followed by headache in 94.54% ( $n = 104$ ), nausea in 68.18% ( $n = 75$ ), myalgia in 66.36% ( $n = 73$ ), vomiting in 62.72% ( $n = 69$ ), retro-orbital pain in 51.81% ( $n = 57$ ), and rash in 2.72% ( $n = 3$ ) [Table 1].

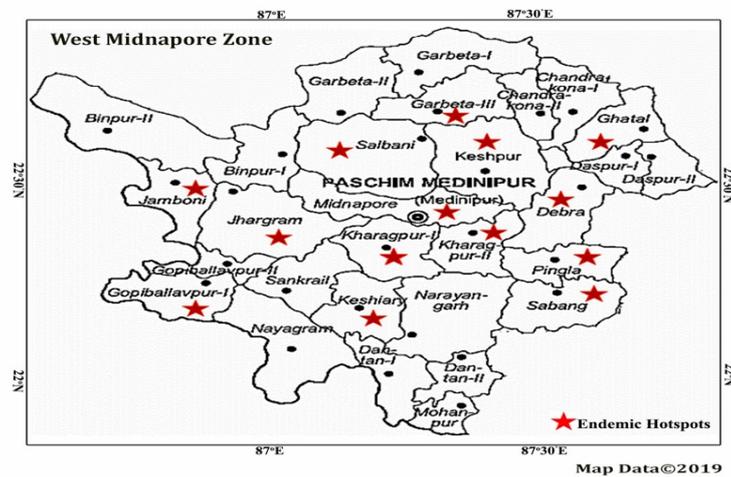
**Table 1:** Frequency of Clinical Symptoms Among Dengue Patients (n = 110)

| Symptom            | Number of Cases (n) | Percentage (%) |
|--------------------|---------------------|----------------|
| Fever              | 110                 | 100            |
| Headache           | 104                 | 94.54          |
| Nausea             | 75                  | 68.18          |
| Myalgia            | 73                  | 66.36          |
| Vomiting           | 69                  | 62.72          |
| Retro-orbital Pain | 57                  | 51.81          |
| Rash               | 3                   | 2.72           |

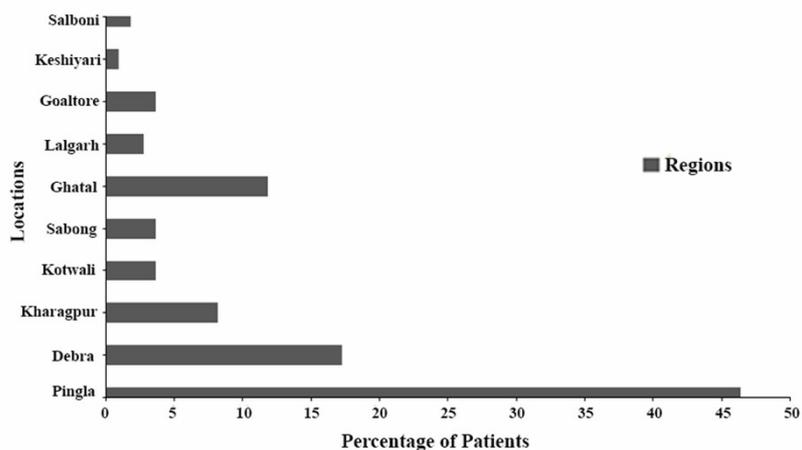
**Demographic overview:**

We found that a large number of patients originated from various rural and suburban zones [Fig. 1], such as Pingla (46.36%, n=51), followed by Debra (19.09%, n=21), Ghatal (11.81%, n=13), Kharagpur (8.18%, n=9), Sabong (3.63%, n=4), and Midnapore Urban (3.63%, n=4), along with other parts of the southern region of West Bengal [Fig. 2].

**Fig 1:** Map of study area and the red star mark zones showing associated endemic zones of dengue in this region



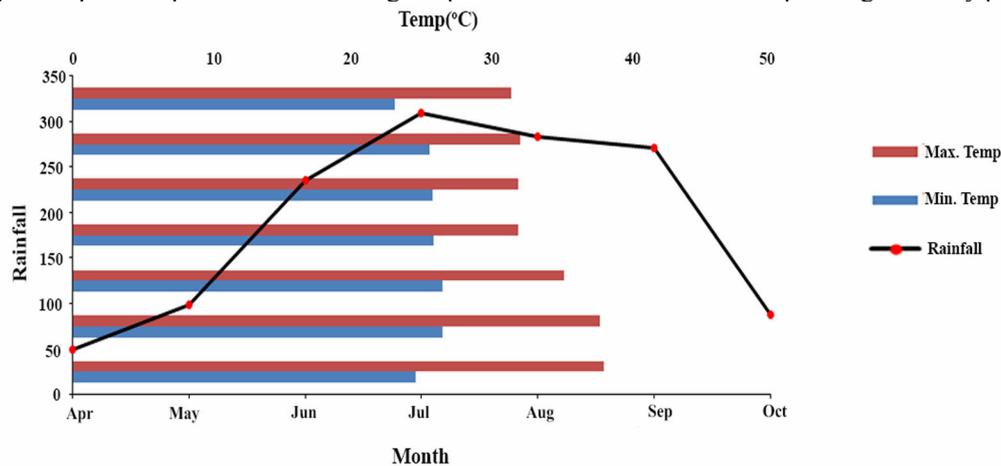
**Fig.2:** Percentage of patients from different locations.



**Weather analysis:**

According to weather data, the average temperatures recorded from April to October 2018 were 31.3°C, 32.1°C, 30.8°C, 28.9°C, 28.8°C, and 27.2°C, respectively. Rainfall during the same period was recorded as 50 mm, 98 mm, 236 mm, 309 mm, 284 mm, 271 mm, and 88 mm, respectively [Fig. 3].

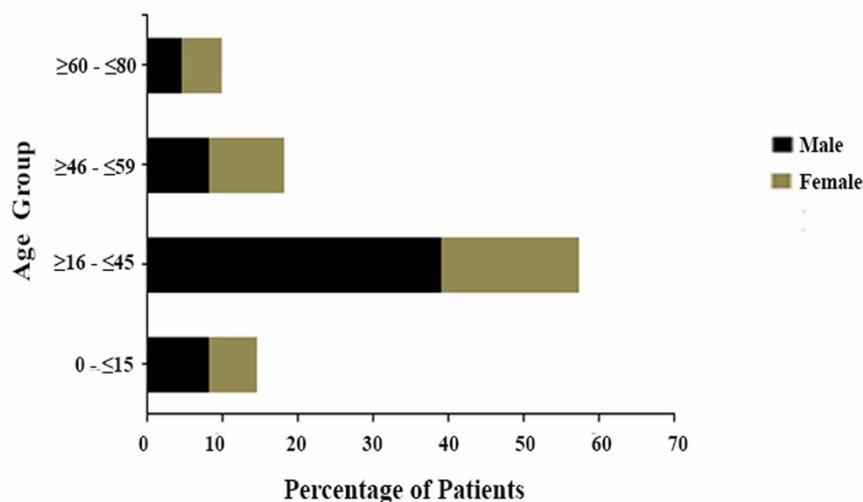
**Fig 3:** Graphical representation showing temperature and rainfall relationship during the study period.



**Age and gender-wise analysis:**

An age group of  $\geq 16$  to  $\leq 59$  years was found to be more susceptible, accounting for 75.45% (n=83) of all confirmed dengue cases. Specifically, the  $\geq 16$  to  $\leq 45$  age group was the most affected, comprising 57.27% (n=63), followed by the  $\geq 46$  to  $\leq 59$  group at 18.18% (n=20), the  $\geq 0$  to  $\leq 15$  group at 14.54% (n=16), and the  $\geq 60$  to  $\leq 80$  group at 10% (n=11). Among the four age groups, the pediatric group (0–15 years) had a mean age of 11.69 with a standard deviation of  $\pm 3.005$  and was statistically insignificant ( $p > 0.1$ ). The younger adult group ( $\geq 16$ –45 years) had a mean age of 27.08 with a deviation of  $\pm 8.453$ , showing statistical significance ( $p < 0.0008$ ). Older adults ( $\geq 46$ –59 years) had a mean age of 51 with a deviation of  $\pm 4.107$ , which was statistically insignificant ( $p > 0.08$ ). Finally, the elderly group ( $\geq 60$ –80 years) had a mean age of 66 with a deviation of  $\pm 6.453$ , and was statistically significant ( $p < 0.2$ ). In the gender-wise distribution of patients, 58.18% (n=64) were male and 41.82% (n=46) were female [Fig. 4].

**Fig 4:** Age and gender wise distribution of the patients.



**Serotyping analysis:**

A total of 110 NS1-positive samples were serotyped using qRT-PCR. Among these, 89.09% (n=98/110) of patients were found to be serotype-positive, with a predominance of the DENV-2 serotype. However, serum from twelve patients tested negative for viral RNA. Based on our observations, a negative DENV qRT-PCR result does not necessarily exclude the presence of the virus. These findings may indicate either a low viral load in the patient or a viral concentration below the detection limit of the assay. Among all the serotyped samples, 86.36% (n=95/110) were positive for DENV-2 RNA, whereas 2.72% (n=3/110) were positive for DENV-3 [Fig. 5].

**Fig 5:** Pie chart showing distribution of dengue virus serotypes (n=110) in an around Southern region of Bengal



#### IV. DISCUSSION

This study aimed to identify the circulating dengue virus (DENV) serotypes in the eastern region of India. Although the first recorded dengue epidemic occurred in Kolkata in 1963–64, comprehensive data on serotype distribution across West Bengal remain scarce (18,19). This region remains a hotspot for several viral diseases, with dengue emerging as the most prevalent in recent years. Recurrent outbreaks have been documented across multiple districts in southern Bengal—including Pingla, Debra, Ghatal, Garbeta, Sabong, Lalgarrh, Goaltore, and Kharagpur—often marked by increasing disease severity, yet limited insights into the circulating DENV serotypes.

During the pre- to post-monsoon study period, we received several samples. After screening, 110 NS1-positive patient sera were selected using a rapid diagnostic kit for inclusion in this study. Environmental factors often serve as key drivers in the emergence and spread of infectious diseases. Dengue epidemics have been reported in several countries during warm, humid, and rainy seasons, as these conditions promote mosquito breeding and shorten the extrinsic incubation period (20). Temperatures ranging from 27°C to 32°C are considered optimal for dengue transmission. Furthermore, rainfall between 50 mm and 309 mm significantly influences disease spread. In our study, a marked increase in rainfall was observed from June to September, aligning with previously reported trends (21).

This clinical and molecular investigation identified Pingla as a potential dengue-endemic hotspot, recording the highest proportion of positive cases (46.36%). Young and active individuals were the primary affected group, particularly those with a history of migration from dengue-endemic regions. Environmental factors and larval survival strategies offer plausible explanations for *Aedes aegypti*-mediated dengue virus transmission. Furthermore, population movement to endemic areas appears to have significantly contributed to the outbreak scenario (22).

Although dengue affects all age groups, previous studies have reported the highest number of cases among individuals aged 5–20 and 21–30 years (23,24). In our study, the greatest impact was observed among individuals aged  $\geq 16$  to  $\leq 45$  years, followed by those aged  $\geq 46$  to  $\leq 59$  years. These age groups primarily comprise working adults and students. Since dengue is predominantly transmitted by day-biting mosquitoes, workplaces and educational institutions likely serve as major hubs of transmission. Following the adult age group, the pediatric population was the next most affected, followed by the geriatric group ( $\geq 60$  to  $\leq 80$  years). A significant difference in NS1 positivity was noted between males and females, with a higher proportion of male patients (58.18%) compared to females (41.82%).

Our study confirms the circulation of two DENV serotypes; however, the prevailing subtypes remain unidentified. The majority of dengue cases were attributed to DENV-2 (86.36%, n=95), aligning with findings from other regions, such as Uttar Pradesh (25). Although DENV-3 (2.72%, n=3) was also detected in certain areas, its prevalence was notably lower than that of DENV-2. No co-infections involving multiple serotypes were observed, which may be due to the limited sample size. A larger sample pool could potentially uncover a more diverse serotype distribution. Notably, a previous study from Ernakulam, Kerala, reported concurrent infections involving multiple dengue virus serotypes (26).

A key question emerging from our study is: Why are Pingla, Debra, and Ghatal more susceptible to dengue outbreaks? One plausible explanation is that these are low-lying areas prone to water stagnation, which

provides ideal breeding conditions for *Aedes* mosquitoes. Additionally, these regions are densely populated, with congested living environments that facilitate rapid virus transmission. Combined, these factors likely contribute to heightened dengue vulnerability and epidemic potential. Other contributing elements—such as rainfall, temperature, humidity, and limited access to timely medical care—may further exacerbate outbreak severity.

The findings of this study are highly significant and represent the first dengue serotyping analysis from this previously unexplored region of eastern India. Few studies have investigated the dengue situation in West Bengal and eastern India at the molecular level. The dengue-endemic zones identified in this research had not been previously examined through molecular methods, and the circulating serotypes were largely unknown (27,28,29). However, it is important to note that serotype dominance can fluctuate over time. Therefore, regular and systematic dengue serotyping surveillance is essential to understand the temporal dynamics of circulating serotypes. This informative study on predominant serotypes will contribute to improved outbreak prediction and preparedness for future dengue epidemics in the region. Further in-depth genotyping of circulating viral strains is strongly recommended to revise public health policies periodically and to help restrict the spread of the dengue virus.

## V. CONCLUSION

This study provides the first molecular evidence of circulating dengue virus serotypes in an underreported region of Southern Bengal, Eastern India. The predominance of DENV-2, along with a limited presence of DENV-3, highlights the urgent need for continued serotype-specific surveillance. Environmental factors, population movement, and socio-demographic patterns were found to influence the spatial and age-specific distribution of dengue cases. The identification of endemic hotspots such as Pingla, Debra, and Ghatal underscores the role of geography and urban density in outbreak dynamics. These findings emphasize the necessity for regular molecular monitoring and targeted public health interventions. Further genotyping studies are recommended to inform vaccine strategies and guide timely updates to local and national dengue control policies.

## CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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